

1. (Twice Amended) A method of determining a nucleotide base in a nucleic acid sample comprising the steps of:

- (i) incubating the nucleic acid sample with a primer, DNA polymerase, and a deoxynucleotide triphosphate, deoxynucleotide triphosphate analogue or a dideoxynucleotide triphosphate, which releases pyrophosphate when added to the primer by action of DNA polymerase;
- (ii) measuring the pyrophosphate released in step (i); and
- (iii) determining that the nucleotide base is complementary to said deoxynucleotide triphosphate, deoxynucleotide triphosphate analogue or dideoxynucleotide triphosphate that is incubated in step (i),

wherein steps (i) to (iii) are performed in a microfluidic device.

2. (Twice Amended) A method for identifying the sequence of a portion of sample DNA comprising the steps of:

- (i) forming immobilised double stranded DNA comprising one strand of sample DNA and one strand of primer DNA on one or more reaction areas in a microchannel structure of a microfluidic device, DNA primers of said one or more reaction areas are hybridised to said sample DNA;
- (ii) adding a deoxynucleotide, deoxynucleotide analogue or dideoxynucleotide and a DNA polymerase to each of said one or more reaction areas so that extension of primer occurs as a result from complementarity of the added deoxynucleotide, deoxynucleotide analogue or dideoxynucleotide with the strand of sample DNA that is part of the immobilised double stranded DNA;
- (iii) detecting whether or not the deoxynucleotide, deoxynucleotide analogue or dideoxynucleotide added in step (ii) is added to the primer DNA in said one or more reaction areas;

- (iv) repeating steps (ii) and (iii) with different deoxynucleotides, deoxynucleotide analogues or dideoxynucleotides the required number of times for the identification of said sequence; and
- (v) identifying said sequence from the results of step (iii).

3. (Twice Amended) A method of determining a nucleotide base in a nucleic acid sample comprising the steps of:

- (i) attaching 0.1 – 200 pmol of a primer DNA or single stranded sample DNA to each of between one and 100,000 pre-determined areas within the surface of a microfluidic device;
- (ii) hybridising single stranded sample DNA to said primer DNA or primer DNA to said single stranded sample DNA, to each of the predetermined areas by utilizing said primer DNA or said single stranded sample DNA that is attached to said predetermined areas in step (i);
- (iii) adding a deoxynucleotide, deoxynucleotide analogue or dideoxynucleotide and a DNA polymerase so that extension of the primer occurs by action of said DNA polymerase with consequent release of pyrophosphate as a result from complementarity for the added deoxynucleotide, deoxynucleotide analogue or dideoxynucleotide with the sample DNA; and
- (iv) measuring the release of pyrophosphate and from which predetermined area it is released thereby enabling determination of said nucleotide base.

4. (Twice Amended) A method for identifying the sequence of a portion of sample DNA, comprising the steps of:

- (i) adding sample DNA to a predetermined area on a microfluidic device;
- (ii) moving the sample DNA to a reaction chamber on the microfluidic device;

- (iii) attaching the sample DNA to a surface of the reaction chamber, wherein a DNA primer is hybridised to the sample DNA in a single stranded form, or hybridising the sample DNA in single stranded form to a DNA primer that is attached to the surface of the reaction chamber;
- (iv) extending the primer in the presence of a DNA polymerase with a deoxynucleotide, deoxynucleotide analogue, or dideoxynucleotide, wherein the extension is indicated by release of pyrophosphate from the extension reaction;
- (v) repeating step (iv) the required number of times for the identification of said sequence; and
- (vi) identifying said sequence from the deoxynucleotides, deoxynucleotide analogues, or dideoxynucleotides that resulted in primer extension in step (iv).

6. (Twice Amended) The method of claim 2, wherein a dideoxynucleotide which is labelled is added in step (ii).

7. (Twice Amended) The method of claim 1, wherein the detection of the deoxynucleotide, deoxynucleotide analogue or dideoxynucleotide added to the primer is performed in real time.

Please add the following new claims:

17. The method of claim 3, wherein steps (iii) and (iv) are repeated with a deoxynucleotide, deoxynucleotide analogue or dideoxynucleotide.

18. A method for identifying a sequence of a portion of sample DNA comprising the steps of:

(i) forming immobilised double stranded DNA comprising one strand of sample DNA and one strand of primer DNA on one or more reaction areas in a microchannel structure of a microfluidic device, the primers of said one or more reaction areas are hybridised to said sample DNA;

(ii) adding fluorescently labelled dideoxynucleotides and a DNA polymerase to each of said one or more reaction areas so that extension of primer occurs as a result from complementarity of the added dideoxynucleotides with the strand of sample DNA that is part of the immobilised double stranded DNA;

(iii) detecting whether or not a deoxynucleotide added in step (ii) is added to the primer DNA in said one or more reaction areas;

(iv) repeating steps (ii) and (iii) with other fluorescently labelled dideoxynucleotides; and

(v) identifying said sequence from the result of step (iii).